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Improvement of Pullulan Production by *Aureobasidium pullulans* under High Cell Density Inoculation Two-Stage Batch Fermentation System

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PULLULAN production by *Aureobasidium pullulans* ATCC 42023 was studied in bioreactor as a high cell density inoculation (HCDI) in two-stage batch culture. Pullulan formation was examined at different agitation rates (100 – 900 rpm) in non-aerated and aerated medium (1.0 vvm) at a range of controlled pH values. Using modified Reeslev & Jensen medium with 5.5 pH, aeration rate of 1.0 vvm and agitation rate of 700 rpm increased pullulan concentration and productivity by 1.73 fold, compared with uncontrolled pH. Under these conditions, maximum pullulan concentration (73.6 gl^{-1}) was obtained after 5 days of fermentation at 28°C. Pullulan yield coefficient relative to biomass, consumed sugar and conversion coefficient after 5 days fermentation period were also increased by about 1.4, 1.07 and 1.14 fold, respectively, comparing with results obtained from aerated medium without controlling initial pH (7.0) at 700 rpm after 4 days of incubation.

Keywords: *A. pullulans* ATCC 42023, (HCDI) Two-stage batch, pH, Pullulan production, Bioreactor.

Pullulan is an extracellular water-soluble microbial polysaccharide produced by strains of *Aureobasidium pullulans*. It is a linear mixed linkage α-D-glucan consisting mainly of maltotriose units interconnected via α-(1→6) linkages (Szymanska & Galas, 1993). A number of potential applications have been reported for this biopolymer as a result of its unique properties. Pullulan is used as a low-calorie ingredient in foods, gelling agent, coating and packaging material for food and drugs, binder for fertilizers and as an oxidation-prevention agent for tablets. Other applications include contact lenses manufacturing, biodegradable foil, plywood, water-solubility enhancer and for enhanced oil recovery (Schuster *et al*., 1993; Israilides *et al*., 1998 and Leathers, 2003). Pullulan production has been a subject of numerous studies conducted under batch culture (Szymanska & Galas, 1993; Madi *et al*., 1997; Roukas, 1999; Szymanska *et al*., 1999; Barnett *et al*., 1999 and Lazaridou *et al*., 2002). Lebrun *et al.* (1994) achieved a maximum pullulan concentration of 35 gl^{-1} after 120 h of batch fermentation using a synthetic medium and a 5 l mechanically stirred tank

reactor. Gibbs (1996) found that in all impeller systems examined, the increase in the agitation rate resulted in a marked and reproducible decrease in exopolysaccharide production by *A. pullulans* under batch conditions in a 10 l stirred tank reactor.

Roukas & Serris (1999) studied the effect of the shear rate on batch pullulan production from beat molasses by *A. pullulans* P56 in an airlift reactor. A maximum polysaccharide concentration (18.5 gl^{-1}), biomass dry weight (14.0 gl^{-1}), polysaccharide yield (38.5 %) and sugar utilization (96 %) was achieved at a shear rate of 42 per sec. Shabatai & Mukmenev (1995) proposed a two-stage process for the production of pigment-free pullulan by *A. pullulans* ATCC 42023. At the first stage, started with pH 4.5 of a medium contained soybean oil as a carbon source and glutamic acid as a nitrogen source, cell mass of about 15 gl^{-1} was obtained. When the soybean oil and glutamic acid were nearly exhausted, cells were shifted to a production medium containing sucrose as the carbon source with continuous nitrogen depletion, where production of pullulan started immediately. During 50 h of the production phase, more than 35 gl^{-1} pullulan was produced. Eltayeb *et al.* (2005) achieved a maximum pullulan concentration (65.3 gl^{-1}) after 5 days of fermentation at 28°C on 7 % corn steep liquor as a sole nitrogen source in modified Reeslev & Jensen medium (20 % sucrose) using shake flasks as a HCDI two-stage batch culture.

Most of publications concerning the control of pullulan synthesis indicated that multiple factors are interacting to regulate pullulan biosynthesis which represents an interesting area for research. In this investigation, pullulan production in relation to conditions like agitation rate, aeration and pH using bioreactor as a HCDI two-stage batch culture was evaluated.

Materials and Methods

Fungal strain

Aureobasidium pullulans ATCC 42023 was obtained from American Type Culture Collection, subcultured on malt agar slants at 30°C, maintained at 4°C, and transferred monthly.

Media

Malt agar medium (Atlas, 1997) was used for propagation and preservation of aureobasidium culture. Modified Reeslev & Jensen medium (20 % sucrose), (Eltayeb *et al.*, 2005), was used for pullulan production after replacement of its nitrogen source with 7% corn steep liquor.

Bioreactor

Fermentation was carried out using a 3 l dished bottom bioreactor Z 6110 / coob (Cole–Parmer Instruments), which consisted of 3 l vessel equipped with lipseal stirrer assembly, automatic pH controller, automatic dissolved O_2, automatic temperature controller.

Experimental techniques

Preparation of standard inoculum

Standard inoculum was prepared by transferring a loop of the tested culture into 250 ml conical flasks containing 50 ml of modified Reeslev & Jensen medium. The inoculated flask was incubated on a rotary shaker at 210 rpm for 48 hr at 30°C. The content of this flask was used as a standard inoculum (1 ml contained 6.0–7.0 x 10^5 viable cells), after first centrifuged at 12000 x g for 15 min, and then cells were washed twice with sterile distilled water and harvested to inoculate productive medium as a HCDI two-stage batch experiments as the method described by Shabtai & Mukmenev (1995).

Fermentation conditions and factors studied

HCDI two-stage batch culture: In this method, the fermentation vessel containing 1800 ml of modified Reeslev & Jensen medium was autoclaved at 121°C for 20 min. The bioreactor was inoculated with washed cells of *A. pullulans* ATCC 42023 prepared as mentioned above. The final working volume was 2 l. Temperature was kept at 28 ± 1°C during cultivation. Speed of agitation was adjusted as required. During fermentation, dissolved O_2 and pH were automatically recorded. Samples (10 – 20 ml) were withdrawn daily from the culture. Biomass dry weight, residual sugars and pullulan concentration were determined in collected samples.

Effect of agitation speed: Two experiments were designed to study the growth behaviour and pullulan production during cultivation at agitation speeds ranged from 100 to 900 rpm without any aeration or with constant aeration rate (1.0 vvm).

Effect of pH: Four pH levels of the production medium, *i.e.*, 2.5, 4.0, 5.5 and 7.0 were automatically controlled by 2 N NaOH addition during the fermentation period.

Pullulan determination: Pullulan was precipitated in the culture supernatant with 2 volumes of ethanol 99 %, at 4°C for 1 h. The precipitate was centrifuged at 4000 x g for 10 min followed by drying overnight at 80°C and was then weighed (GÖksungur *et al.*, 2004).

Chemical determinations: Total residual sugars were determined in the fermented liquor according to the method described by Flood & Priestly (1973).

Parameters related to pullulan production: Yield factor (%), pullulan yield coefficient relative to biomass ($Y_{p/x}$), conversion coefficient (%), pullulan yield (%) and productivity (P) were calculated according to Gamal *et al.* (1991). Correlation coefficient and regression analysis were carried out according to Microsoft Excel (Microsoft Corporation, 2006).

Results and Discussion

A. pullulans ATCC 42023 was cultivated in modified Reeslev & Jensen medium using bioreactor as a two-stage batch culture in order to evaluate the effect of some operating conditions such as agitation, aeration and pH in relation to pullulan production.

Effect of agitation speed

Different oxygen transfer rates were examined in two experiments of aerated and non-aerated culture by using different agitation speeds for the production medium.

In non-aerated cultures

Results of the effects of different agitation speeds on cell dry weight and pullulan production in modified Reeslev & Jensen medium without air supply in stirred bioreactor during 6 days production was recorded in Tables 1 & 2. Data clearly show that cell dry weight of the tested strain was increased during fermentation period to record the highest value after 5, 4 and 3 days at low (100, 300 rpm), moderate (500 rpm) and high agitation speeds (> 500 rpm), respectively. Pullulan production gave the same trend at low agitation speeds, whereas, the highest figures were observed at 500, 700 and 900 rpm after 6, 6 and 5 days, respectively. Increasing the agitation speeds led to an increase in the amount of both cell dry weight and produced pullulan up to maximum concentration, *i.e.*, 23 and 42.6 gl^{-1} at 900 rpm after 3 and 5 days fermentation period, respectively. The production of pullulan at different agitation speeds was high during 72 – 96 hr of fermentation particularly with 900 rpm.

The highest consumed sugar (103.5 gl^{-1}) was observed after 6 days at 900 rpm. The highest figures of pullulan yield 21.1% and productivity 0.38 $gl^{-1}h^{-1}$ were recorded at this treatment after 6 and 4 days, respectively. The highest conversion coefficient (45.5 %) was observed at 100 rpm after 5 days of fermentation period. A highly positive correlation coefficient (0.986) between agitation speed and pullulan concentration was observed, indicating a strong relationship between dissolved oxygen and amount of produced pullulan. It was also noticed that air saturation levels obtained by different speeds at the start of production stage ranged from 35 – 87 % and then rapidly decreased to 3.1 or 3.2 at the end of fermentation period. This may reduce oxygen transfer rate to the cells even at high agitation rate (900 rpm). Therefore, a second experiment was conducted to study the effect of air supply on pullulan production at different agitation rates.

In aerated cultures

Data given in Tables 3 & 4 show the effect of different agitation speeds on pullulan production in modified Reeslev & Jensen medium supplemented with constant aeration rate (1.0 vvm air) during 6 days of fermentation period. Data revealed that both cell dry weight and pullulan concentration gave the highest values after 6 days at 300 & 500 rpm and after 5 days at 700 & 900 rpm agitation rates, respectively. These treatments approximately recorded the same value of growth yield factor (%). Moreover, the value 2.3 gg^{-1} of $Y_{p/x}$ was similarly observed after 6 days in culture agitated with 300 or 500 rpm and it was also reached to 2.6 gg^{-1} after 5 days with 700 & 900 rpm agitation rate.

TABLE 1. Effect of different agitation rates on pullulan production by *A. pullulans* ATCC 42023 in modified Reeslev & Jensen non - aerated medium during 6 days of incubation at 28°C using bioreactor as a HCDI two-stage batch culture.

Incubation time (hr)	Agitation rate (rpm)																								
	100					300					500					700					900				
	Cell dry weight (gl^{-1})	Pullulan concentration (gl^{-1})	$Y_{p/x}$ (gg^{-1})	Consumed sugar (gl^{-1})	Air saturation (%)	Cell dry weight (gl^{-1})	Pullulan concentration (gl^{-1})	$Y_{p/x}$ (gg^{-1})	Consumed sugar (gl^{-1})	Air saturation (%)	Cell dry weight (gl^{-1})	Pullulan concentration (gl^{-1})	$Y_{p/x}$ (gg^{-1})	Consumed sugar (gl^{-1})	Air saturation (%)	Cell dry weight (gl^{-1})	Pullulan concentration (gl^{-1})	$Y_{p/x}$ (gg^{-1})	Consumed sugar (gl^{-1})	Air saturation (%)	Cell dry weight (gl^{-1})	Pullulan concentration (gl^{-1})	$Y_{p/x}$ (gg^{-1})	Consumed sugar (gl^{-1})	Air saturation (%)
24	2.2	1.2	0.54	6.12	11	2.41	2.11	0.88	10.5	16	3.32	4.2	1.27	19	22	4.68	6.5	1.39	25.6	31	5.1	8.2	1.61	29.7	39
48	2.7	2.2	0.81	7.8	3.5	5.76	4.2	0.73	14.2	5.3	7.2	6.3	0.88	18.7	6.7	13.5	9.8	0.73	28.7	15.1	13.6	14.2	1.04	41	25.3
72	3.1	6.7	2.16	22.8	3.2	10.3	10.1	0.98	33.6	3.3	13.2	13.4	1.02	40.3	4.4	22.6	15.6	0.69	44.3	7.5	23	24.2	1.05	68.2	10.8
96	3.6	16.7	4.64	46	3.2	15.4	21.3	1.38	56.9	3.2	19.3	29.4	1.52	76.7	3.1	21.4	32.2	1.5	80.9	4.1	22.5	36.3	1.61	94	5.3
120	4.0	21.0	5.25	47.2	3.1	18.1	28.4	1.57	67.2	3.2	19.2	35.1	1.83	82.6	2.3	20.1	38.2	1.9	89.3	3.1	21.2	42.6	2	101.8	3.2
144	3.7	19.19	5.2	55.9	3.1	17.9	28.1	1.57	72.5	3.2	18.24	37.2	2.04	89.6	3.2	20.1	39.2	1.9	93.8	3.3	21.3	42.2	2	103.5	3.4

Air saturation at zero time were: 35, 43, 52, 76 and 87 %, for 100, 300, 500, 700 and 900 rpm, respectively. Cell dry weight at zero time were: 2.0, 2.2, 2.6, 2.7 and 2.6 gl^{-1} for 100, 300, 500, 700 and 900 rpm, respectively.

TABLE 2. Pullulan production related parameters as influenced by different agitation speeds in modified Reeslev & Jensen non-aerated medium during 6 days of incubation at 28°C using bioreactor as a HCDI two-stage batch culture.

Incubation time (hr)	Agitation rate (rpm)																								
	100					300					500					700					900				
	Pullulan concentration (gl^{-1})	Consumed sugar (gl^{-1})	Conversion coefficient (%)	Pullulan yield (%)	Productivity ($gl^{-1}h^{-1}$)	Pullulan concentration (gl^{-1})	Consumed sugar (gl^{-1})	Conversion coefficient (%)	Pullulan yield (%)	Productivity ($gl^{-1}h^{-1}$)	Pullulan concentration (gl^{-1})	Consumed sugar (gl^{-1})	Conversion coefficient (%)	Pullulan yield (%)	Productivity ($gl^{-1}h^{-1}$)	Pullulan concentration (gl^{-1})	Consumed sugar (gl^{-1})	Conversion coefficient (%)	Pullulan yield (%)	Productivity ($gl^{-1}h^{-1}$)	Pullulan concentration (gl^{-1})	Consumed sugar (gl^{-1})	Conversion coefficient (%)	Pullulan yield (%)	Productivity ($gl^{-1}h^{-1}$)
24	1.2	6.12	19.6	0.6	0.05	2.11	10.5	20.1	1.05	0.09	4.2	19	22.1	2.1	0.17	6.5	25.6	25.4	3.2	0.27	8.2	29.7	27.6	4.1	0.34
48	2.2	7.8	28.2	1.1	0.05	4.2	14.2	29.5	2.1	0.09	6.3	18.7	33.6	3.15	0.13	9.8	28.7	34.1	4.9	0.2	14.2	41	34.6	7.1	0.29
72	6.7	22.8	29.4	3.35	0.09	10.1	33.6	30.1	5.05	0.14	13.4	40.3	33.2	6.7	0.19	15.6	44.3	35.2	7.8	0.22	24.2	68.2	35.5	12.1	0.34
96	16.7	46	36.3	8.35	0.17	21.3	56.9	37.4	10.7	0.22	29.4	76.7	38.4	14.7	0.31	32.2	80.9	39.8	16.1	0.34	36.3	94	38.6	18.1	0.38
120	21.0	47.2	44.5	10.5	0.18	28.4	67.2	43.3	14.2	0.24	35.1	82.6	42.4	17.5	0.29	38.2	89.3	42.7	19.1	0.32	42.6	101.8	41.8	20.8	0.36
144	19.2	55.9	34.3	9.6	0.13	28.1	72.5	38.8	14	0.19	37.2	89.6	41.5	18.6	0.26	39.2	93.8	41.8	19.6	0.27	42.2	103.5	40.8	21.1	0.29

TABLE 3. Effect of different agitation rates on pullulan production by *A. pullulans* ATCC 42023 in modified Reeslev & Jensen aerated (1 vvm) medium during 6 days of incubation at 28°C using bioreactor as a HCDI two-stage batch culture.

Incubation time (hr)	Agitation rate (rpm)																							
	300						500						700						900					
	Cell dry weight (gl^{-1})	Pullulan conc. (gl^{-1})	$Y_{p/x}$ (gg^{-1})	Yield factor (%)	Consumed sugar (gl^{-1})	Air saturation (%)	Cell dry weight (gl^{-1})	Pullulan conc. (gl^{-1})	$Y_{p/x}$ (gg^{-1})	Yield factor (%)	Consumed sugar (gl^{-1})	Air saturation (%)	Cell dry weight (gl^{-1})	Pullulan conc. (gl^{-1})	$Y_{p/x}$ (gg^{-1})	Yield factor (%)	Consumed sugar (gl^{-1})	Air saturation (%)	Cell dry weight (gl^{-1})	Pullulan conc. (gl^{-1})	$Y_{p/x}$ (gg^{-1})	Yield factor (%)	Consumed sugar (gl^{-1})	Air saturation (%)
24	2.51	6.3	2.5	0.5	21.8	32	3.44	8.4	2.44	2.8	26.6	45	4.89	15	3	5.3	39.6	57.6	5.66	12.3	2.2	9.2	33.2	74.2
48	3.6	9.6	2.7	3.7	32.8	18.8	5.6	12.5	2.2	7.5	38.9	25.1	7.6	24.8	3.3	7.3	65.2	41.6	9.3	22.2	2.4	11.4	59	49
72	5.0	18.3	3.7	4.3	60	6.3	7.21	24.1	3.3	6.8	66.2	7.8	10.5	39.4	3.8	8	97.3	16	12.52	36.2	2.9	10.6	93.8	26
96	7.7	39.1	5	4.5	117	4.6	8.5	45.6	5.4	4.9	119.2	5.8	22.2	60.1	2.7	13.5	144.2	7.5	21	57.2	2.72	12.8	143.7	9.2
120	15.81	41.3	2.6	11.4	117.3	3.2	17.1	46.2	2.7	12	120	3.2	23.1	60.8	2.6	13.9	145.9	4.6	22.33	57.3	2.6	13.8	142.9	5.3
144	18.6	42	2.3	13.7	118.3	3.2	21.2	48.6	2.3	15	122.7	3.2	22	60.8	2.7	13	147.4	3.3	20.5	56.6	2.8	12.4	144.8	4.2

Zero time air saturation 73, 86, 95 and 100 (%), for 300, 500, 700 and 900 rpm, respectively. Zero time cell dry weight: 2.4, 2.7, 2.8 and 2.6 (gl^{-1}) for 300, 500, 700 and 900 rpm, respectively.

TABLE 4. Pullulan production related parameters of as influenced by different agitation rates in modified Reeslev & Jensen aerated (1 vvm) medium during 6 days of incubation at 28°C using bioreactor as a HCDI two-stage batch culture.

Incubation time (hr)	Agitation rate (rpm)																			
	300					500					700					900				
	Pullulan conc. (gl^{-1})	Consumed sugar (gl^{-1})	Conversion coefficient (%)	Pullulan yield (%)	Productivity ($gl^{-1}h^{-1}$)	Pullulan conc. (gl^{-1})	Consumed sugar (gl^{-1})	Conversion coefficient (%)	Pullulan yield (%)	Productivity ($gl^{-1}h^{-1}$)	Pullulan conc. (gl^{-1})	Consumed sugar (gl^{-1})	Conversion coefficient (%)	Pullulan yield (%)	Productivity ($gl^{-1}h^{-1}$)	Pullulan conc. (gl^{-1})	Consumed sugar (gl^{-1})	Conversion coefficient (%)	Pullulan yield (%)	Productivity ($gl^{-1}h^{-1}$)
24	6.3	21.8	28.9	3.15	0.26	8.4	26.6	31.6	4.2	0.35	15	39.6	37.9	7.5	0.62	12.3	33.2	37.1	6.15	0.51
48	9.6	32.8	29.3	4.8	0.2	12.5	38.9	32.1	6.25	0.26	24.8	65.2	38	12.4	0.51	22.2	59	37.6	11.1	0.46
72	18.3	60	30.5	9.15	0.25	24.1	66.2	36.4	12	0.33	39.4	97.3	40.4	19.7	0.54	36.2	93.8	38.6	18.7	0.5
96	39.1	117	33.4	19.55	0.4	45.6	119.2	38.3	22.8	0.48	60.1	144.2	41.7	30	0.63	57.2	143.7	39.8	28.6	0.59
120	41.3	117.3	35.2	20.65	0.34	46.2	120	38.5	23.1	0.39	60.8	145.9	41.7	30.4	0.51	57.3	142.9	40.1	28.6	0.48
144	42	118.3	35.5	21	0.29	48.6	122.7	39.6	24.3	0.33	60.8	147.4	40.9	30.4	0.42	56.6	144.8	39.1	28.3	0.39

The highest figures of cell dry weight and pullulan concentration, *i.e.*, 23.1 & 60.8 gl^{-1} were obtained at 700 rpm agitation. This indicates that the tested strain behaved similarly with respect to growth and pullulan production at agitation rates lower or higher than 500 rpm with constant air supply. At agitation rates 700 & 900 rpm, little increase in pullulan concentration and conversion coefficient was detected with increasing fermentation period from 4 to 5 days. The highest pullulan production related parameters, *i.e.*, 60.1 gl^{-1}, 41.6%, 30% and 0.63 $gl^{-1}h^{-1}$ for pullulan concentration, conversion coefficient, pullulan yield and productivity were obtained after 4 days in aerated culture at 700 rpm, respectively.

Comparing the highest amount of pullulan produced in aerated cultures to that in unaerated culture at different agitation rates, it is interesting to notice that pullulan concentration and productivity were increased by 1.41 & 1.85 fold due to aeration treatment. Also, using 1.0 vvm air supply was reduced the agitation rate and fermentation time needed for effective pullulan production to 700 rpm and 4 days, respectively as illustrated in Fig. 1. These results indicated the importance of oxygen for pullulan production. Oxygen was reported to be essential for pullulan production because *A. pullulans* is an aerobic organism, and oxygen was thought to play a critical role (Rho *et al*., 1988). Whoever, other authors considered that high oxygen concentration could affect pullulan production, (McNeil & Kristiansen, 1990 and Wecker & Onken, 1991). Rho *et al*. (1988) stated that high pullulan yield and synthesis rate are associated with high oxygen tension in the growth medium. Also, Audet *et al*. (1996) reported that higher dissolved oxygen concentration generally led to higher productivity. They added that the optimum pullulan yield could be achieved at an intermediate dissolved oxygen concentration. Using higher volumetric air flow rate led to increase biomass and pullulan production.

Effect of pH

Tables 5 & 6 illustrate the data of pullulan production at different controlled levels of pH with aeration rate of 1.0 vvm and agitation rate of 700 rpm using bioreactor as HCDI two-stage batch technique. At the end of fermentation period (6 days), no considerable variation in cell dry weight was observed at pH values ranged from 2.5 to 5.5, whereas, the highest cell yield factor (71.8%) was obtained at pH 2.5, then decreased sharply with increasing the pH to 4.0. This could be attributed to increasing the sugar consumption rate without an increase in biomass formation. Therefore, it could be stated that biomass formation was favored at low pH. On contrast, pullulan production and consumed sugar were increased with the increase in pH level to reach the maximum figures (73.6 and 157 gl^{-1}) at pH 5.5 after 5 and 6 days fermentation period, respectively. Increasing in pH level more than 5.5 led to decrease in all pullulan parameters. At pH 5.5, the highest figures of conversion coefficient, pullulan yield and productivity, *i.e.*, 47.5 %, 36.8 % and 0.61$gl^{-1}h^{-1}$, respectively, were recorded after 5 days. Statistical analysis revealed a high positive correlation coefficient between controlled pH values ranged from 2.5-5.5 and both produced pullulan and consumed sugar being 0.97 and 0.93, respectively.

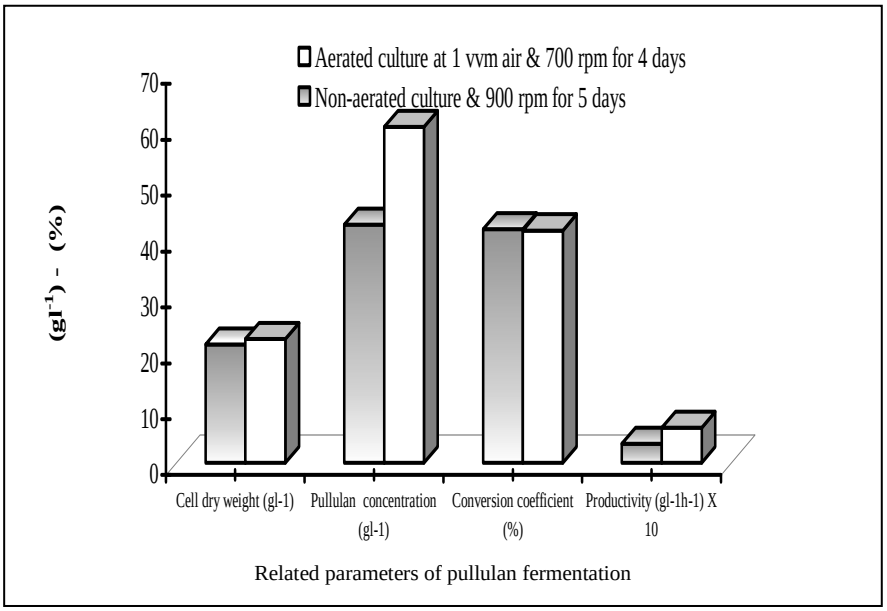

Fig. 1. Comparison between pullulan production of *A. pullulans* ATCC 42023 in aerated and non-aerated cultures at different agitation rates in modified Reeslev & Jensen medium after 5 days of incubation at 28°C using bioreactor as a HCDI two-stage batch culture.

Comparing the previous results with that obtained from aerated medium without controlling the initial pH (7.0) at 700 rpm after 4 days (Tables 3 & 4), it could be observed that under controlled pH of 5.5, pullulan concentration, $Y_{p/x}$, consumed sugar and conversion coefficient after 5 days fermentation period were increased by about 1.22, 1.4, 1.07 and 1.14 fold, respectively, on the other hand, cell dry weight decreased by 10.4% as shown in Fig. 2. In this respect, Lacroix *et al.* (1985) found that the highest pullulan production in the second stage of fermentation was attained by adjusting the medium pH to 5.5. Also, McNeil & Kristiansen (1990) and Wanru *et al*. (1995) stated that the optimum pH for both biomass and pullulan production was 4.5.

From the aforementioned data of pullulan production, it could be concluded that using bioreactor as a HCDI two-stage batch culture under controlled pH of 5.5, aeration rate of 1.0 vvm and agitation rate of 700 rpm increased pullulan concentration and productivity by 1.73 fold, than similar condition without controlling the pH (Table 2). Statistical analysis revealed a high positive correlation coefficient between incubation time and each of cell dry weight, pullulan formation or consumed sugar being 0.98 under that batch fermentation, therefore, it is recommended to use bioreactor with this system for pullulan production by *A. pullulans* ATCC 42023 after 5 days fermentation period using modified Reeslev & Jensen medium.

TABLE 5. Cell dry weight and pullulan production by *A. pullulans* ATCC 42023 as influenced by different controlled pH levels in modified Reesley & Jensen medium with aeration rate at 1.0 vvm and agitation rate at 700 rpm during 6 days of incubation at 28°C using bioreactor as a HCDI two-stage batch culture.

Incubation time (hr)	pH levels															
	2.5				4.0				5.5				7.0			
	Cell dry weight (gl^{-1})	Pullulan conc. (gl^{-1})	$Y_{p/x}$ (gg^{-1})	Yield factor (%)	Consumed sugar (gl^{-1})	Cell dry weight (gl^{-1})	Pullulan conc. (gl^{-1})	$Y_{p/x}$ (gg^{-1})	Yield factor (%)	Consumed sugar (gl^{-1})	Cell dry weight (gl^{-1})	Pullulan conc.(gl^{-1})	$Y_{p/x}$ (gg^{-1})	Yield factor (%)	Consumed sugar (gl^{-1})	Cell dry weight (gl^{-1})	Pullulan conc.(gl^{-1})	$Y_{p/x}$ (gg^{-1})	Yield factor (%)	Consumed sugar (gl^{-1})
24	4.1	2.1	0.51	22.9	7	3.5	5.4	1.54	5.3	16.8	3.2	8.7	2.72	2.9	20.2	3.5	6.3	1.8	5.6	17.7
48	6.6	2.3	0.35	55.4	7.4	4.4	9.4	2.1	7	25.4	4.2	25.6	6.09	2.9	55.5	4.3	17.6	4.09	4	44.8
72	8.9	6.4	0.72	33.5	19.1	7.8	18.2	2.3	10.7	48.4	9.3	39.5	4.25	7.9	84.4	6.6	27.4	4.15	6	68
96	14.8	8.2	0.55	53	23.2	13.8	28.2	2.04	15.1	74	13.6	56.4	4.14	9.2	119.5	10.2	32.3	3.16	9.8	78.4
120	18.8	9.6	0.51	63	25.9	19.1	55.2	2.89	12.1	136.2	19.9	73.6	3.7	11.2	154.9	16.6	44.3	2.67	13.4	105.5
144	20.1	9.2	0.45	71.8	24.5	19.5	56.1	2.88	12.2	138.3	20.2	73	3.6	11.2	157	17.4	43.5	2.5	14	106.4

Cell dry weight at zero time was: 2.5, 2.6, 2.6 and 2.5 (gl^{-1}) for pH values: 2.5, 4.0, 5.5 and 7.0, respectively.

TABLE 6. Pullulan production related parameters as influenced by different controlled pH levels in modified Reesley & Jensen medium with aeration rate at 1.0 vvm and agitation rate at 700 rpm during 6 days of incubation at 28°C using bioreactor as a HCDI two-stage batch culture.

Incubation time (hr)	pH levels															
	2.5				4.0				5.5				7.0			
	Pullulan conc. (gl^{-1})	Consumed sugar (gl^{-1})	Conversion coefficient (%)	Pullulan yield (%)	Productivity ($gl^{-1}h^{-1}$)	Pullulan conc.(gl^{-1})	Consumed sugar (gl^{-1})	Conversion coefficient (%)	Pullulan yield (%)	Productivity ($gl^{-1}h^{-1}$)	Pullulan conc. (gl^{-1})	Consumed sugar (gl^{-1})	Conversion coefficient (%)	Pullulan yield (%)	Productivity ($gl^{-1}h^{-1}$)	Pullulan conc.(gl^{-1})	Consumed sugar (gl^{-1})	Conversion coefficient (%)	Pullulan yield (%)	Productivity ($gl^{-1}h^{-1}$)
24	2.1	7	30	1.05	0.088	5.4	16.8	32.1	2.7	0.225	8.7	20.2	43	4.35	0.36	6.3	17.7	35.6	3.15	0.26
48	2.3	7.4	31	1.15	0.048	9.4	25.4	37	4.7	0.19	25.6	55.5	46.1	12.8	0.53	17.6	44.8	39.3	8.8	0.36
72	6.4	19.1	33.5	3.2	0.089	18.2	48.4	37.6	9.1	0.25	39.5	84.4	46.8	19.75	0.55	27.4	68	40.3	13.7	0.38
96	8.2	23.2	35.3	4.1	0.085	28.2	74	38.1	14.1	0.29	56.4	119.5	47.2	28.2	0.59	32.3	78.4	41.2	16.1	0.34
120	9.6	25.9	37	4.8	0.08	55.2	136.2	40.5	27.6	0.46	73.6	154.9	47.5	36.8	0.61	44.3	105.5	42	22.15	0.37
144	9.2	24.5	37.6	4.6	0.06	56.1	138.3	40.6	28	0.39	73	157	46.5	36.5	0.5	43.5	106.4	40.9	21.8	0.3

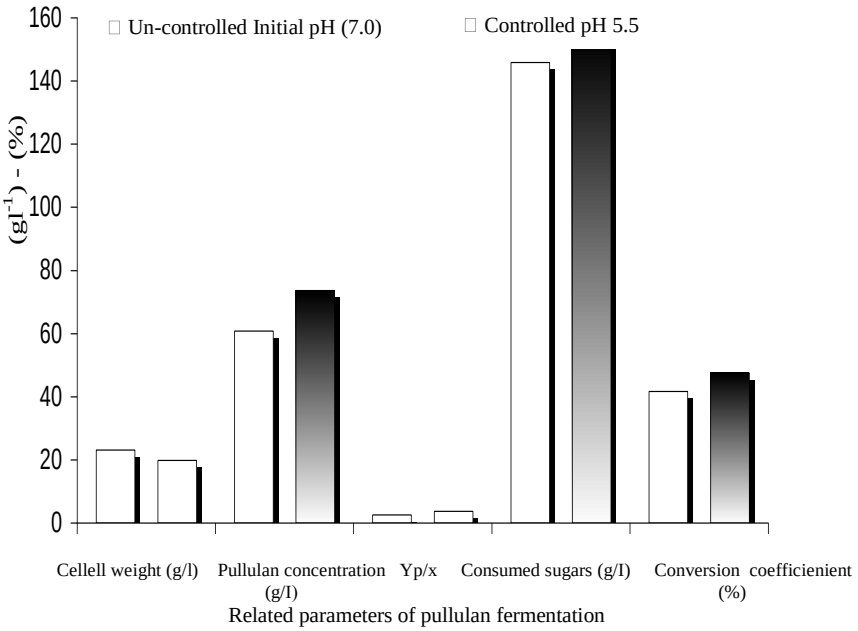

Fig. 2. Comparison between pullulan production by *A. pullulans* ATCC 42023 in modified Reeslev & Jensen medium, aeration rate of 1 vvm, agitation rate of 700 rpm, controlled pH of 5.5 and uncontrolled initial pH of 7.0 after 5 days of incubation at 28°C using bioreactor as a HCDI two-stage batch culture.

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تحسين انتاج البوليولان بواسطة فطر *Aureobasidium pullulans* بالتخمير بنظام الدفعة الواحدة ذات المرحلتين مع التلقيح بتركيز عالي للخلايا

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أجرى هذا البحث لدراسة امكانية تحسين انتاج البوليولان بواسطة السلالة *Aureobasidium pullulans* ATCC 42023 و ذلك بالتنمية في المخمر بنظام الدفعة الواحدة ذات المرحلتين و الملقحة بتركيز عالي للخلايا. حيث تم اختبار إنتاج البوليولان عند مستويات مختلفة من معدلات التقليب تراوحت بين ١٠٠ – ٩٠٠ لفة/ق فى البيئة المهواه أو غير المهواه عند مستويات مختلفة من الحموضة المتحكم فيها. و قد أظهرت النتائج أن التنمية باستخدام بيئة ريسلف و جنسن عند حموضة ثابتة مقدارها ٥,٥ و معدل تهوية مقداره ١,٠vvm و معدل تقليب مقداره ٧٠٠ لفة/ق زاد تركيز البوليولان و إنتاجيته بمقدار ١,٧٣ ضعفاً مقارنة بنفس النظام بدون تحكم في درجة الحموضة . كما وجد أن أعلى إنتاج للبوليولان (٧٣,٦ جم/لتر) كان بعد خمسة أيام من التحضين على درجة حرارة ٢٨°م عند تطبيق الظروف السابقة. أزدادت تحت هذه الظروف قيم كل من: $Y_{p/x}$, السكر المستهلك و معامل التحويل بعد خمسة أيام من التحضين حيث بلغت ١,٤, ١,٠٧ و١,١٤ مرة على الترتيب و ذلك مقارنة بالنتائج المتحصل عليها من البيئة المهواه وغيرالمتحكم في درجة الحموضة الخاصة بها و التي كانت عند بداية التخمر تساوى ٧,٠ و معدل تقليب ٧٠٠ لفة/ق و ذلك بعد أربعة أيام من التحضين.